

Resolving T cell – T cell transfer of HIV-1 by optical nanoscopy

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The Acquired Immune Deficiency Syndrome (AIDS) is caused by the Human Immunodeficiency Virus (HIV). A thorough understanding of the infection on a cellular level is important for the development of new classes of therapeutics or vaccines to combat this virus.

HIV can enter CD4⁺ T cells either as a cell-free virus or by direct cell-cell transmission. An infected T cell can engage in a virus dependent adhesive structure with an uninfected CD4 + T cell, called the Virological Synapse (VS), where virus assembly occurs preferentially within a spatially narrowly confined region and where newly synthesized virus is endocytosed by the target cell. This process occurs on a small spatial scale, while the cells are highly polarized and motile [1, 2].

Resolving the viral transfer involves optical nanoscopy methods that can follow this process with high resolution and high speed. Our method of choice is super-resolved Structured Illumination Microscopy (SR-SIM) which provides double the resolution compared to conventional microscopy and has the ability to simultaneously acquire fast, super-resolved three-dimensional images, with multiple colors. [3].

The simultaneous observation of several components of the infection process is possible].

For our experiments, Jurkat-T cells were transfected with plasmids encoding replication competent infectious fluorescent HIV. Fluorescent proteins are expressed as fusions to components of the virus such as Gag and Env (Fig. 1). Uninfected, primary CD4⁺ T cells were added to the virus-expressing Jurkat-T cells. In these studies, we demonstrate that SR-SIM can resolve single virus particles at the T cell-to-T cell VS. SR-SIM presents a powerful tool for future live cell studies on the propagation of HIV through endocytosis at the VS.

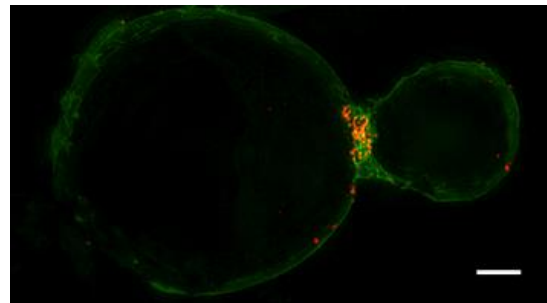


Figure 1: SR-SIM image of a virological synapse between a Jurkat-T cell and primary T cell. Red: Gag-iCherry, green: plasma membrane. Scale bar, 2 μ m.

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